

## The Genus *Bitylenchus* Filip'ev, 1934 (Nematoda: Tylenchida) with Descriptions of Two New Species from Spain

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**ABSTRACT:** The taxonomic position of the genus *Bitylenchus* Filip'ev, 1934, is discussed and its diagnosis emended. Two new species, *B. serranus* sp. n. and *B. pratensis* sp. n., from southeastern Spain are described and illustrated using light and scanning electron microscopy (SEM). *Bitylenchus serranus* sp. n. is characterized by a hemispherical lip region with 6–8 annuli, stylet 21  $\mu$ m long, lateral field with regularly areolated outer bands, a short postanal intestinal sac extending up to one-fourth of the tail length, and a broadly conoid tail usually ventrally curved (about 90% of specimens studied). *Bitylenchus pratensis* sp. n. is characterized by a subcylindrical female tail regularly tapering to a rounded and striated terminus and a basal esophageal bulb as long as or longer than the isthmus. Several populations of *B. maximus* (Allen, 1955) Siddiqi, 1986, from Scotland, Spain, Trinidad, and U.S.A. are described and compared. The Spanish population is characterized by the presence of males and inseminated females; SEM observations are provided. A population of *B. huesingi* (Paetzold, 1958) Siddiqi, 1986, is described. A table with the morphometric data of each species is included.

**KEY WORDS:** *Bitylenchus huesingi*, *Bitylenchus maximus*, *Bitylenchus pratensis* sp. n., *Bitylenchus serranus* sp. n., plant-parasitic nematodes, SEM observations, Spain, taxonomy.

During a nematode survey on several plant communities in southeastern Spain, 2 new and 2 known species of the genus *Bitylenchus* Filip'ev, 1934 (Siddiqi, 1986), were collected. Females and males of the 4 species are described herein as *B. serranus* sp. n., *B. pratensis* sp. n., *B. maximus* (Allen, 1955) Siddiqi, 1986, and *B. huesingi* (Paetzold, 1958) Siddiqi, 1986. Morphology of 3 female populations of *B. maximus* from Trinidad, U.S.A., and Scotland is reported. Morphometry and morphology using SEM observations are also given.

### Materials and Methods

Nematodes were killed by gentle heat and fixed in a 4% solution of formaldehyde, then dehydrated and processed to glycerine according to Seinhorst's method. Body length and curved structures were measured with the aid of a precision curvimeter; straight structures, such as maximum body width, stylet, anal body width, etc., were measured using a micrometer-scale in the eyepiece of a high-power ( $\times 1,250$ ) microscope. All measurements are in micrometers unless otherwise stated.

For examination under the scanning electron microscope (SEM), specimens were processed with Spurr's low viscosity epoxy resin, coated with gold (De Grisse, 1973), and examined with a Zeiss DSM 950 scanning electron microscope at accelerating voltages of 10 and 15 kV.

### Description

#### *Bitylenchus serranus* sp. n. (Table 1, Figs. 1–14)

**HOLOTYPE** (female in glycerine):  $L = 883 \mu\text{m}$ ;  $a = 32.7$ ;  $b = 6.1$ ;  $b_1 = 10.3$ ;  $V = 53$ ;  $c = 17.3$ ;  $c' = 2.4$ ; stylet =  $22 \mu\text{m}$ ;  $m = 53$ ;  $S = 1.5$ ;  $O = 9$ ; tail annuli = 40.

**PARATYPE FEMALES** ( $N = 36$ ): Morphometrics are given in Table 1. Body moderately thin, slightly curved ventrally upon relaxation. Body annuli opposite mid-esophagus 1–2 and opposite mid-body 1 wide. Lateral field with 4 lines, (8–11) wide at mid-body, and outer bands areolated. Lip region hemispherical, anteriorly flattened, set off by a slight constriction from the body; 4 high and 7–9 wide, bearing 6–8 annuli. Labial plate fused with labial sectors (Figs. 10, 11). Longitudinal indentations on lip annuli behind amphids present (Figs. 10, 11). Cephalic framework lightly sclerotized. Stylet 2.5–2.7 times longer than lip region width, conus 11–12 long. Basal knobs rounded, anterior surface posteriorly sloping, flattened, or slightly indented, 4–5 across. Dorsal esophageal gland orifice 2 from stylet base. Procorpus slender, 1.1–1.6 times longer than isthmus. Median esophageal bulb round-oval, strongly muscular, 13–17 long  $\times$  10–14 wide,

Table 1. Morphometric data for *Bitylenchus serranus* sp. n. female and male paratypes (measurements in  $\mu\text{m}$ ).

	Females (N = 36)		Males (N = 18)	
	$\bar{x} \pm \text{SD}$	Range	$\bar{x} \pm \text{SD}$	Range
L	824.0 $\pm$ 47.3	734.0–922.0	756.0 $\pm$ 61.4	650.0–906.0
a	30.0 $\pm$ 1.5	26.0–33.0	32.0 $\pm$ 2.1	28.0–36.0
b	6.0 $\pm$ 0.5	5.0–7.0	5.0 $\pm$ 0.4	5.0–6.0
b <sub>1</sub>	10.0 $\pm$ 0.8	8.0–12.0	10.0 $\pm$ 0.5	8.0–10.0
V%	52.0 $\pm$ 1.5	49.0–54.0	—	—
T%	—	—	48.0 $\pm$ 5.7	39.0–58.0
G <sub>1</sub>	25.0 $\pm$ 3.4	18.0–32.0	—	—
G <sub>2</sub>	27.0 $\pm$ 3.5	19.0–34.0	—	—
c	17.0 $\pm$ 1.5	14.0–20.0	15.0 $\pm$ 1.4	13.0–19.0
c'	2.6 $\pm$ 0.2	2.0–3.0	2.7 $\pm$ 0.2	2.5–3.0
Stylet length	21.0 $\pm$ 0.6	19.0–22.0	20.0 $\pm$ 0.6	19.0–21.0
m	52.0 $\pm$ 0.9	50.0–55.0	52.0 $\pm$ 1.2	50.0–53.0
o	10.0 $\pm$ 1.1	9.0–13.0	11.0 $\pm$ 1.1	10.0–13.0
S	1.4 $\pm$ 0.3	1.0–2.0	1.5 $\pm$ 4.2	1.4–1.5
MB	51.0 $\pm$ 1.6	49.0–55.0	52.0 $\pm$ 1.7	49.0–55.0
Nerve ring	96.0 $\pm$ 7.5	79.0–113.0	91.0 $\pm$ 7.2	79.0–104.0
Excretory pore	121.0 $\pm$ 9.2	97.0–137.0	113.0 $\pm$ 8.0	101.0–128.0
Esophagus length	145.0 $\pm$ 10.9	116.0–161.0	137.0 $\pm$ 9.8	124.0–156.0
Maximum body width	28.0 $\pm$ 1.4	25.0–31.0	24.0 $\pm$ 1.9	19.0–27.0
Anal body width	19.0 $\pm$ 1.5	16.0–22.0	19.0 $\pm$ 1.0	17.0–20.0
Tail length	50.0 $\pm$ 5.4	40.0–62.0	52.0 $\pm$ 4.1	45.0–57.0
Tail annuli	38.0 $\pm$ 4.3	31.0–46.0	—	—
Spicule length	—	—	28.2 $\pm$ 1.4	26.0–31.0
Gubernaculum length	—	—	14.2 $\pm$ 0.7	13.0–16.0

with refractive cuticular valve 4 long. Basal bulb pyriform, 21–29 long, 12–15 wide. Excretory pore located opposite anterior half of basal bulb level. Hemizonid occupying 3–4 annuli, located 2–4 annuli anterior to excretory pore. Esophago–intestinal junction with 2 cardinal cells. Fasciculi (lateral canal, serpentine canal) present, occasionally obscure.

Vulva transverse, flush with body surface or slightly raised; body cuticle just anterior to vulva slightly thickened; body just behind vulva slightly constricted. Vagina straight, 10–12 long. Spermatheca round, 12–18 wide, filled with round sperm, 2–3 wide. Egg in uterus oval, 18–19  $\times$  60–61. Ovaries outstretched, posterior usually longer than anterior, with a single row of oocytes. Tail subcylindroid, with rounded terminus, and usually ventrally curved (about 90% of specimens studied), rarely straight. Terminus coarsely annulated, terminal cuticle 5–9 thick. Short post-anal intestinal sac 10–19 long, extending up to one-fourth of tail length. Phasmids porelike, located anterior to middle of tail, and at 7–17 annuli posterior to anus level, seldom opposite.

ALLOTYPE (male in glycerine): L = 805  $\mu\text{m}$ ; a = 31.0; b = 5.8; T = 52; c = 14.6; c' = 2.7;

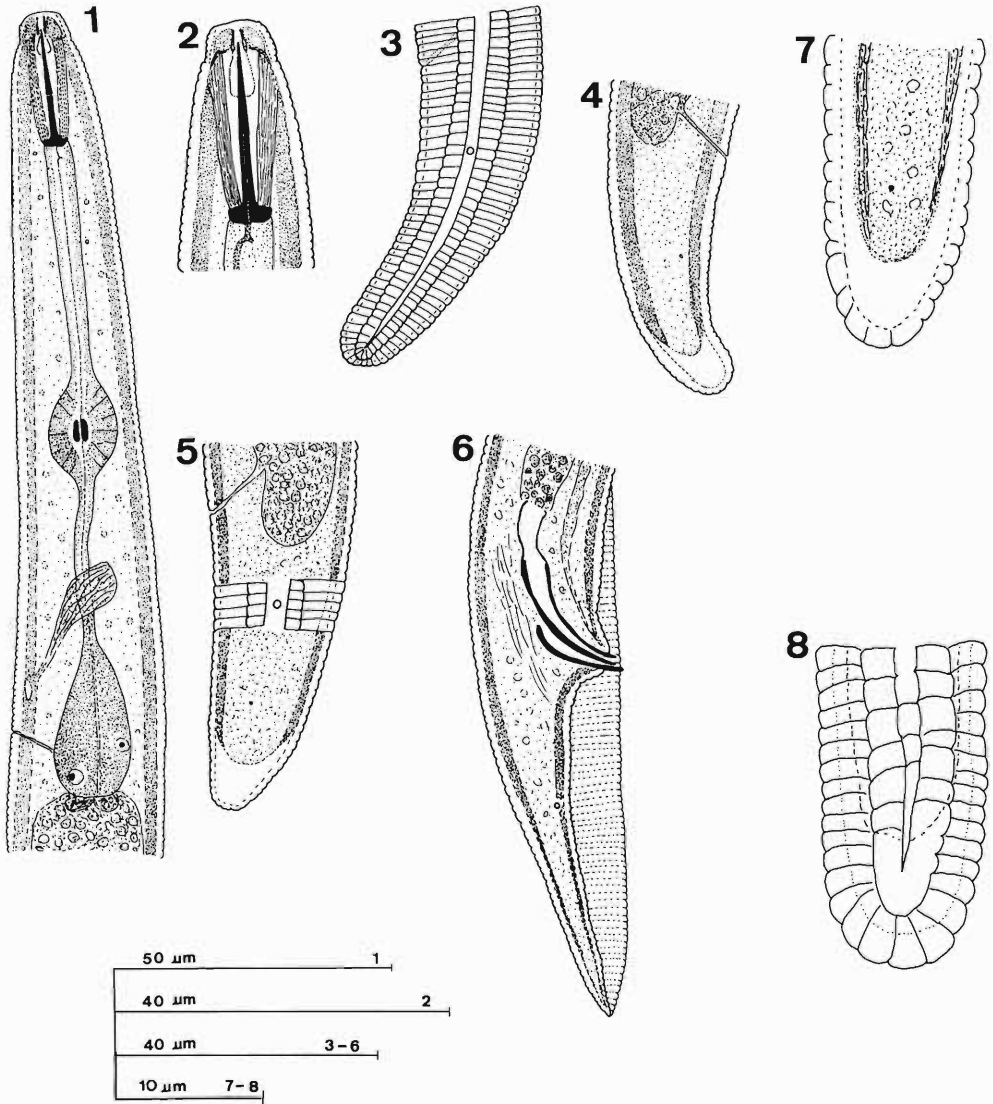
stylet = 19  $\mu\text{m}$ ; m = 52; S = 1.5; O = 10; spicules = 30  $\mu\text{m}$ ; gubernaculum = 14  $\mu\text{m}$ .

PARATYPE MALES (N = 18): Morphometrics are given in Table 1. Similar to female in measurements and morphology, except for the sexual dimorphism of tail region. Testis well developed, with 2 rows of spermatogonia, 301–499 long. Spicules ventrally curved, with pointed tip. Gubernaculum bent, not protruding. Tail conoid, enveloped by a crenate bursa 80–103 long. Phasmid located 16–26 from anus.

TYPE HABITAT AND LOCALITY: Specimens collected around the roots of pinus (*Pinus nigra salzmanni*) at Nava del Espino, Sierra de Cazorla, Jaén, Spain.

TYPE SPECIMENS: Holotype female, allotype male, 29 female and 15 male paratypes (slides BTC 1–BTC 28) at nematode collection of Centro de Investigación y Desarrollo Agrario, Granada, Spain; 6 female and 2 male paratypes (slides BTC 24–BTC 28) at CAB International Institute of Parasitology (CIP), St. Albans, Herts., England.

DIAGNOSIS AND RELATIONSHIPS: *Bitylenchus serranus* sp. n. is characterized by a hemispherical lip region with 6–8 annuli, stylet 19–23 long,



Figures 1-8. *Bitylenchus serranus* sp. n. 1. Female anterior region. 2. Lip region. 3-5. Female tails. 6. Male tail. 7, 8. Tail termini of females.

lateral field with regularly areolated outer bands, a short postanal intestinal sac extending up to one-fourth of tail length, and tail subcylindroid, usually ventrally curved, rarely straight.

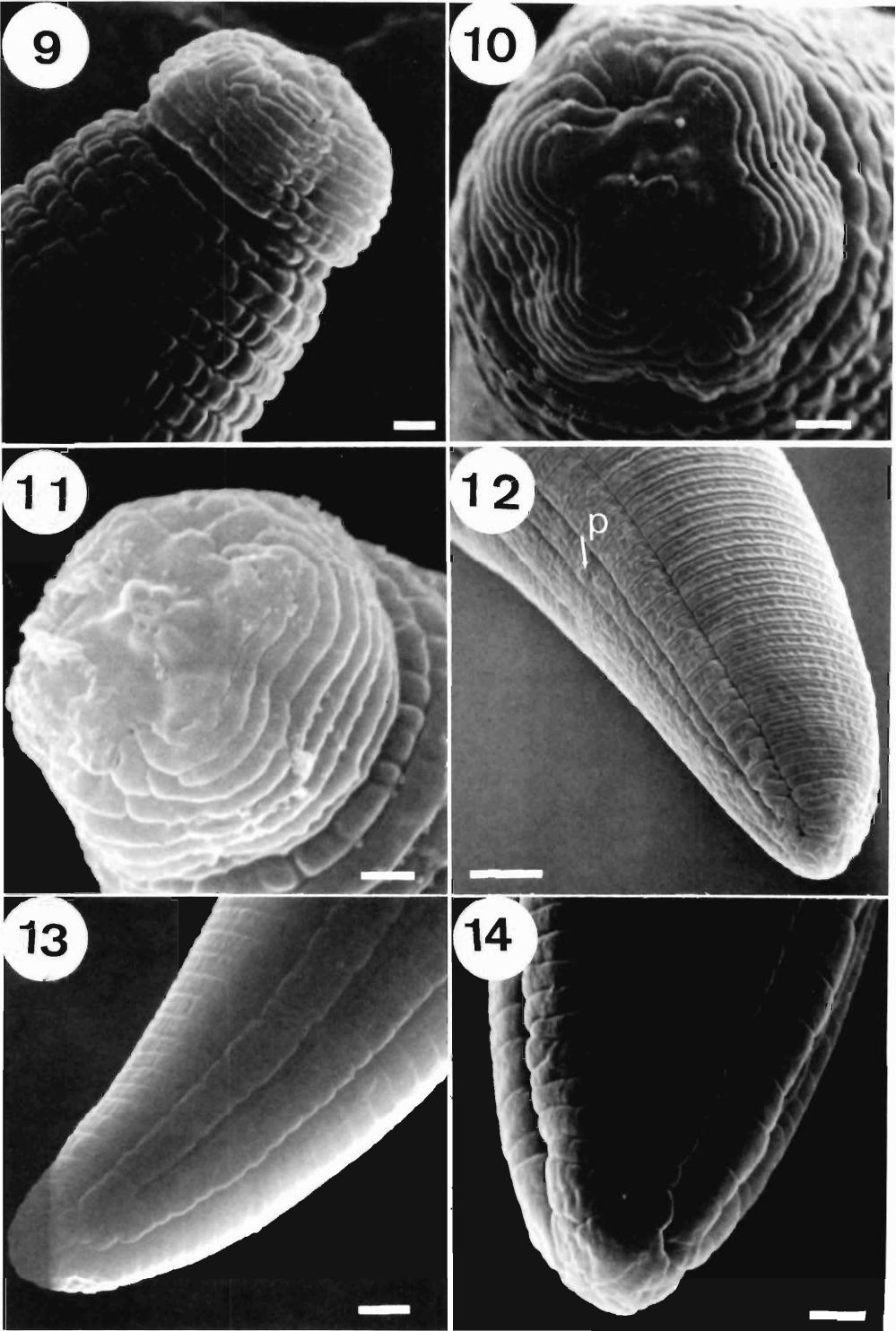
*Bitylenchus serranus* is most similar to *B. canalis* and *B. wilskii*. It differs from *B. canalis* in having the excretory pore located opposite the anterior half of the basal bulb vs. opposite the esophageal base; a short postanal intestinal sac extending up to one-fourth of the tail length vs. long and completely filling tail cavity, and female tail with fewer than 50 annuli and terminus being

coarsely annulated. It differs from *B. wilskii* in finer body annuli: 1 vs. 2-3 wide, in stylet length: 19-23 vs. 24-27, larger stylet knobs, and female tail terminus being coarsely annulated.

The SEM face view is quite similar to that of *B. tobari* as it is shown by Sauer (1985).

***Bitylenchus pratensis* sp. n.**  
(Table 2, Figs. 15-28)

HOLOTYPE (female in glycerine): L = 1,070  $\mu$ m; a = 34.5; b = 6.4; b<sub>1</sub> = 10.2; V = 53; c =



Figures 9–14. SEM micrographs of *Bitylenchus serranus* sp. n. 9. Lip region. 10, 11. Face views. 12, 13. Tail region. 14. Tail terminus. Scale bars: 9–11 = 1  $\mu$ m; 12, 13 = 5  $\mu$ m; 14 = 2  $\mu$ m. p, phasmid.

**Table 2.** Morphometric data for *Bitylenchus pratensis* sp. n. female and male paratypes (measurements in  $\mu\text{m}$ ).

	Females ( $N = 18$ )		Males ( $N = 6$ )	
	$\bar{x} \pm \text{SD}$	Range	$\bar{x} \pm \text{SD}$	Range
L	1,106.0 $\pm$ 127.8	828.0–1,296.0	1,033.0 $\pm$ 35.3	979.0–1,078.0
a	36.9 $\pm$ 3.9	29.9–46.3	41.8 $\pm$ 3.1	37.3–45.2
b	7.5 $\pm$ 0.8	5.5–9.0	6.9 $\pm$ 0.4	6.5–7.4
b <sub>1</sub>	11.7 $\pm$ 1.0	10.0–13.8	11.5 $\pm$ 0.5	11.1–12.1
V%	52.0 $\pm$ 1.5	50.0–53.0	—	—
T%	—	—	43.0 $\pm$ 17	24.0–64.0
G <sub>1</sub>	28.0 $\pm$ 2.9	22.0–31.0	—	—
G <sub>2</sub>	26.0 $\pm$ 4.0	18.0–30.0	—	—
c	18.3 $\pm$ 1.9	14.8–22.7	19.0 $\pm$ 1.3	17.5–21.2
c'	2.8 $\pm$ 0.2	2.6–3.4	3.0 $\pm$ 0.1	2.9–3.1
Stylet length	20.5 $\pm$ 0.6	20.0–22.0	20.3 $\pm$ 0.4	20.0–21.0
m	55.0 $\pm$ 2.2	53.0–60.0	54.0 $\pm$ 1.7	53.0–56.0
o	10.7 $\pm$ 1.4	9.5–12.2	12.2 $\pm$ 4.6	9.5–17.5
S	1.5 $\pm$ 0.1	1.4–1.5	1.5 $\pm$ 0.1	1.4–1.6
MB	55.0 $\pm$ 2.1	52.0–59.0	54.0 $\pm$ 2.3	51.0–55.0
Nerve ringe	101.0 $\pm$ 9.8	83.0–115.0	102.0 $\pm$ 7.8	92.0–107.0
Excretory pore	129.0 $\pm$ 12.7	108.0–149.0	127.0 $\pm$ 4.0	123.0–131.0
Esophagus length	148.0 $\pm$ 12.4	124.0–168.0	150.0 $\pm$ 9.9	137.0–162.0
Maximum body width	30.0 $\pm$ 4.3	24.0–37.0	25.0 $\pm$ 1.7	23.0–27.0
Anal body width	21.6 $\pm$ 2.7	17.0–26.0	18.2 $\pm$ 1.0	17.0–19.0
Tail length	61.0 $\pm$ 6.1	48.0–70.0	54.0 $\pm$ 3.1	49.0–57.0
Tail annuli	28.0 $\pm$ 2.7	24.0–33.0	—	—
Spicule length	—	—	34.0 $\pm$ 0.8	33.0–35.0
Gubernaculum length	—	—	15.3 $\pm$ 1.0	14.0–16.0

16.7; c' = 2.7; stylet = 21  $\mu\text{m}$ ; m = 53; S = 1.4; O = 9.5; tail annuli = 28.

**PARATYPE FEMALES ( $N = 18$ ):** Morphometrics are given in Table 2. Body ventrally arcuate to an open C-shaped; annuli prominent, 1.5–2.0 wide near mid-body. Lateral field 9–12 wide at mid-body; with 4 incisures, outer ones crenate; outer bands regularly areolated. Lip region conoid-rounded, clearly set off, 7–8 wide  $\times$  4–5 high, with 5–6 distinct annuli and an inconspicuous labial disc. SEM face view shows a squarish labial disc and that the anteriormost cephalic annulus is divided into 6 sectors (Fig. 27), and 6 longitudinal grooves on other lip annuli. Cephalic framework lightly sclerotized. Stylet 1.4–1.5 times as long as lip region width, with small rounded posteriorly sloping knobs 3–3.5 across. Dorsal esophageal gland orifice 2–3 from stylet base. Median esophageal bulb oval 14–19 long, with refractive valve 5–6 long. Basal bulb saccate to pyriform, 19–31 in length and as long as or longer than isthmus. Esophago-intestinal valve well developed, rounded, 5–7 long, positioned slightly into base of esophagus. Excretory pore just behind hemizonid or 1–3 annuli posterior to it, usually opposite anterior half of basal bulb.

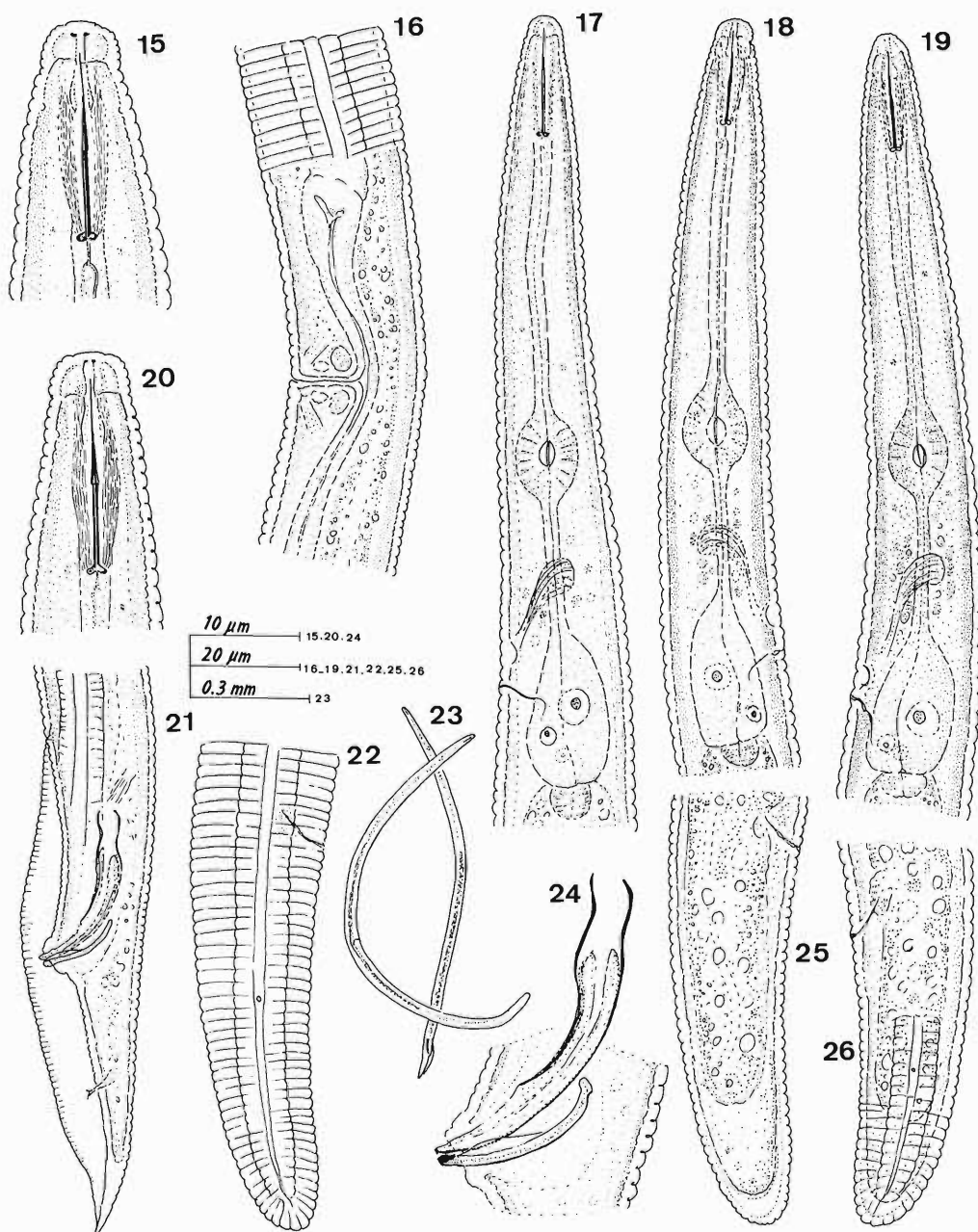
Vulva transverse; vagina straight, 13–17 long.

Ovaries outstretched, anterior usually longer than posterior, with a single row of oocytes, except 2 in multiplication zone. Spermatheca spherical, 16–18 wide, filled with rounded sperm 2.5–3 wide. Egg in uterus oval, 31 wide  $\times$  93 long. Intestine with fasciculi and refractive globules. Postanal extension of intestine extending  $\frac{2}{3}$  to  $\frac{3}{4}$  of tail length. Tail subcylindrical, regularly tapering to a small rounded, striated terminus. Terminal hyaline portion 5–8 long. Phasmids large, 9–13 annuli posterior to level of anus.

**ALLOTYPE** (male in glycerine): L = 1,039  $\mu\text{m}$ ; a = 40.0; b = 7.2; T = 37; c = 21.2; c' = 2.9; stylet = 20  $\mu\text{m}$ ; m = 53; S = 1.4; O = 17.5; spicules = 34  $\mu\text{m}$ ; gubernaculum = 16  $\mu\text{m}$ .

**PARATYPE MALES ( $N = 6$ ):** Morphometrics are given in Table 2. Similar to female in most details. Body usually straight to slightly ventrally curved. Testis outstretched. Bursa crenate, enveloping tail, 89–108 long. Spicules arcuate, pointed; gubernaculum arcuate and not protrusible, with a distinct velum. Tail conoid, enveloped by a large bursa. Phasmid located 24–27 posterior to anus.

**TYPE HABITAT AND LOCALITY:** Specimens collected from soil in a grassy field at Sierra Morena, Andujar, Jaén, Spain.



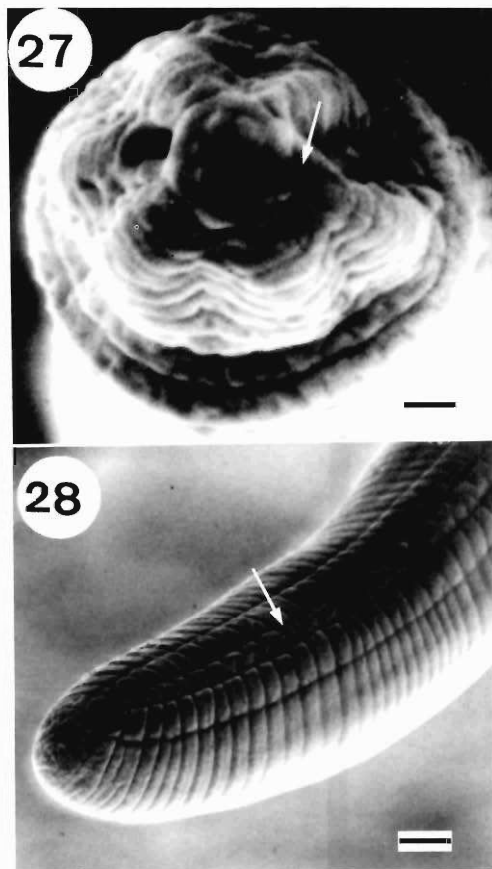
Figures 15–26. *Bitylenchus pratensis* sp. n. 15. Female head region. 16. Vulva region and lateral field. 17–19. Female esophageal regions. 20. Male head region. 21. Male tail terminus. 22, 25, 26. Female tails. 23. Female and male in death posture. 24. Spicule and gubernaculum.

**TYPE SPECIMENS:** Holotype female, allotype male, 7 female and 2 male paratypes (slides BTA 10–BTA 14) at nematode collection of Centro de Investigación y Desarrollo Agrario, Granada, Spain; 10 female and 4 male paratypes (slides

BTA 16–BTA 21) at CAB International Institute of Parasitology (CIP), St. Albans, Herts., England.

**DIAGNOSIS AND RELATIONSHIPS:** *Bitylenchus pratensis* sp. n. is characterized by a conoid





Figures 27, 28. SEM micrographs of *Bitylenchus pratensis* sp. n. 27. Face view. 28. Tail region. Scale bars: 27 = 1  $\mu$ m; 28 = 5  $\mu$ m.

rounded lip region, with 5–6 distinct annuli; stylet 20–21 long; lateral field with regularly areolated outer bands; and a subcylindrical tail, regularly tapering to a small, rounded, striated terminus.

*Bitylenchus pratensis* sp. n. most closely resembles *B. maximus* and *B. serranus*. It differs from *B. maximus* in body posture (ventrally arcuate to an open C-shaped vs. C-shaped to loose spiral); female tail shape (subcylindrical, regularly tapering to a small rounded terminus vs. cylindrical with broadly rounded terminus with fewer annuli in terminal hyaline region); basal esophageal bulb (as long as or longer than isthmus vs. clearly shorter than isthmus, see Figs. 29–35). From *B. serranus* it differs in lip region shape (conoid-rounded vs. hemispherical); smaller stylet knobs; tail shape (subcylindrical,

straight vs. broadly conoid, usually ventrally curved); labial disc in SEM observations (squarish labial disc and anterior cephalic annulus divided into 6 sectors vs. labial disc fused with labial sectors; Figs. 15 and 10, 11, respectively), and spicule length (26–31 vs. 33–35).

*Bitylenchus maximus* (Allen, 1955)

Siddiqi, 1986

(Table 3, Figs. 29–41)

**FEMALE:** Body C-shaped to loose spiral. Lateral field 9–13 wide at mid-body, with 4 lines, outer bands regularly areolated along body and inner band irregularly areolated at tail region (Fig. 41). Lip region hemispherical with flattened anterior end; 7–8 wide  $\times$  4–5 high. There are some differences in the lip region between Spanish and other populations. Spanish specimens (bisexual population) lips set off from body and have 7–9 annuli between anterior end of body to end of outer extension of cephalic framework; whereas U.S.A. and Scotland specimens are slightly set off and have 10–11 annuli. Labial disc and anterior cephalic annulus divided into 6 sectors (Figs. 37, 38). Longitudinal grooves on lip annuli behind amphids (Fig. 37). Stylet attenuated, with posteriorly inclined basal knobs. Procorpus cylindrical, 43–65 long. Median esophageal bulb oval, 16–19 long, strongly muscular with refractive valve 5.5–6 in length. Excretory pore located opposite the beginning of basal bulb. Hemizonid 2–3 annuli long, located 2–3 annuli anterior to excretory pore.

Ovaries outstretched, equally developed. Spermatheca round 13–18 wide, filled with rounded sperm (2–2.5 wide) in Spanish population only. Fasciculi well developed. Postanal intestinal sac long. Phasmid located 11 (9–14) annuli posterior to anus. Tail cylindrical with length different between Spanish and the other populations (see *c'* values in Table 3). Anus a rounded pore (Fig. 39). Terminal hyaline region 6–8 long.

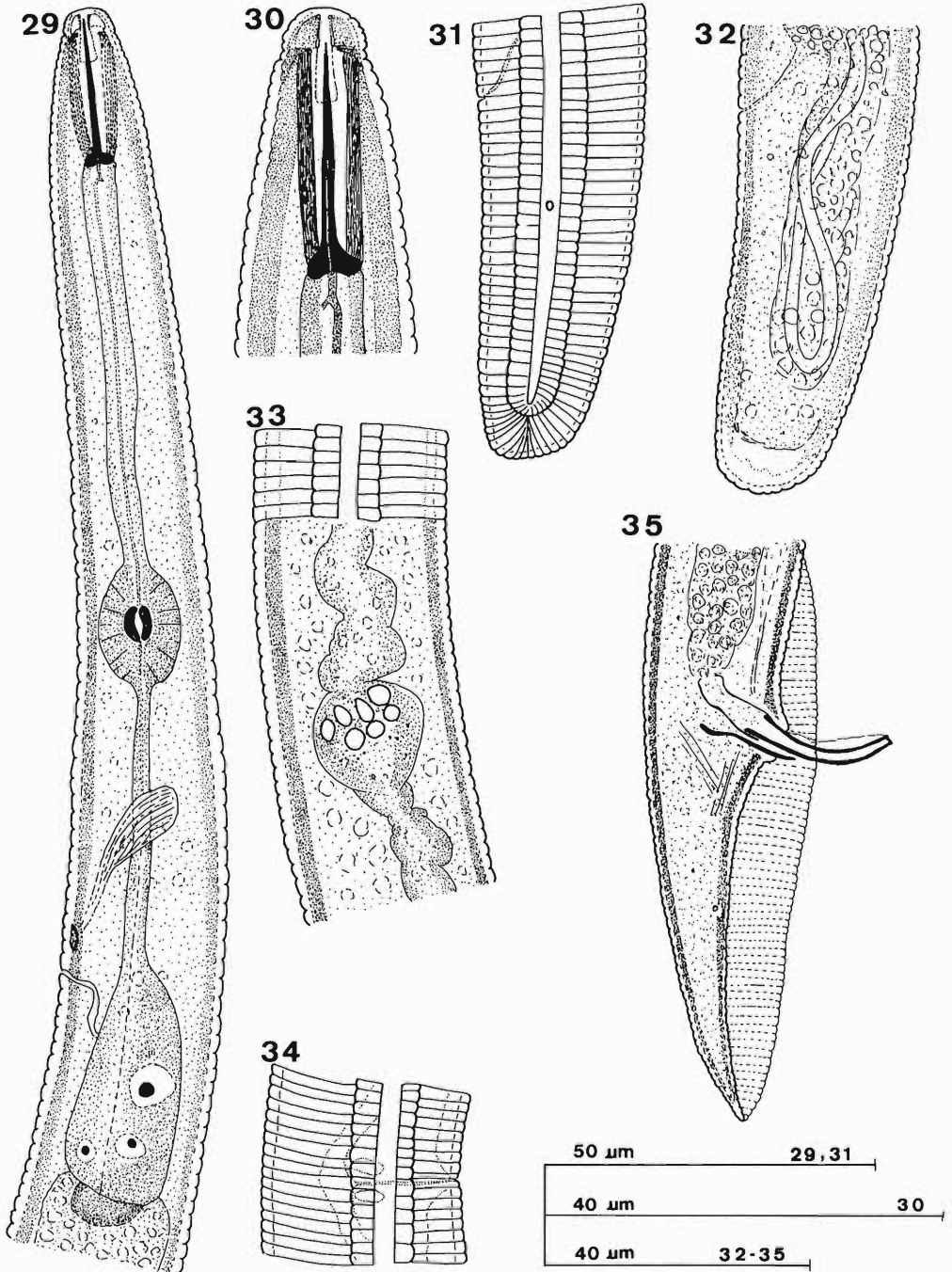
**MALE:** Relatively common in Spanish population but absent in the populations from U.S.A., Trinidad, and Scotland. Similar to female in general morphology and measurements except in a slight difference in size of the lip region (lower in males), 6.5–7 wide  $\times$  3.5–4 high. Gubernaculum with a slight proximal curvature. Bursa crenate, surrounds tail completely, 86–108 long. Phasmid 23–30 from anus.

**HABITAT AND LOCALITIES:** For Spanish population same as for *B. serranus* sp. n. Other localities are from trees by river bank in Minne-

Table 3. Morphometric data for different populations of *Britylencius maximus* (Allen, 1955) Siddiqi, 1986.

	Spain				U.S.A				Scotland		Trinidad	
	Females (N = 31)		Males (N = 12)		Females (N = 4)		Females (N = 5)		Females (N = 5)		Females (N = 2)	
	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range
L	1,278.0 $\pm$ 162.0	1,031.0-1,617.0	935.0 $\pm$ 67.8	797.0-1,023.0	1,125.0 $\pm$ 83.0	1,019.0-1,212.0	1,236.0 $\pm$ 48.0	1,191.0-1,294.0	1,172.0-1,328.0			
a	42.6 $\pm$ 4.3	33.9-51.2	36.2 $\pm$ 2.5	31.9-40.0	45.5 $\pm$ 3.4	43.1-50.5	42.5 $\pm$ 2.2	40.1-44.9	42.8-43.4			
b	7.6 $\pm$ 0.7	6.4-9.2	5.9 $\pm$ 0.6	4.9-7.0	6.9 $\pm$ 0.3	6.6-7.3	7.5 $\pm$ 0.3	7.1-8.0	6.5-7.5			
b <sub>1</sub>	12.6 $\pm$ 1.3	10.0-15.5	9.9 $\pm$ 1.0	8.6-11.7	11.8 $\pm$ 0.8	11.2-12.9	12.7 $\pm$ 0.4	12.2-13.3	10.2-11.8			
V%	52.0 $\pm$ 1.6	50.0-56.0	—	—	51.5 $\pm$ 2.4	50.0-55.0	48.4 $\pm$ 2.5	44.0-50.0	50.0			
T%	—	—	50.0 $\pm$ 6.5	38.0-61.0	—	—	—	—	—			
G <sub>1</sub>	22.0 $\pm$ 3.9	14.0-27.0	—	—	19.0 $\pm$ 3.0	16.0-23.0	20.0 $\pm$ 1.4	19.0-21.0	—			
G <sub>2</sub>	21.0 $\pm$ 3.7	12.0-26.0	—	—	19.0 $\pm$ 3.9	15.0-24.0	19.0	—	—			
c	22.3 $\pm$ 2.1	18.5-27.3	17.2 $\pm$ 2.0	13.7-19.6	18.0 $\pm$ 0.9	16.7-18.8	18.0 $\pm$ 1.3	16.2-19.4	17.5-18.0			
c'	2.5 $\pm$ 0.3	2.0-3.1	2.7 $\pm$ 0.3	2.5-3.3	3.6 $\pm$ 0.4	3.3-4.1	3.3 $\pm$ 0.3	3.1-3.8	3.0-3.4			
Stylet length	22.0 $\pm$ 1.0	20.0-23.0	21.3 $\pm$ 1.0	20.0-23.0	21.0	—	23.0 $\pm$ 1.5	21.0-24.5	21.0-22.5			
m	53.0 $\pm$ 2.0	50.0-57.0	55.0 $\pm$ 1.7	52.0-57.0	56.7 $\pm$ 1.5	56.0-59.0	57.0 $\pm$ 1.7	56.0-59.0	56.0			
o	9.0 $\pm$ 1.3	8.0-13.0	11.0 $\pm$ 1.6	9.0-13.0	9.5	—	10.0	—	2.0-2.5			
s	1.4 $\pm$ 0.1	1.1-1.6	1.5 $\pm$ 0.05	1.4-1.6	1.4 $\pm$ 0.1	1.3-1.6	1.4	—	1.4-1.5			
MB	54.0 $\pm$ 2.0	51.0-58.0	54.0 $\pm$ 1.9	51.0-57.0	52.3 $\pm$ 0.5	52.0-53.0	54.0 $\pm$ 1.7	51.0-55.0	57.0-58.0			
Nerve ring	116.0 $\pm$ 9.8	98.0-136.0	107.0 $\pm$ 9.3	89.0-120.0	106.0 $\pm$ 3.8	101.0-109.0	107.0 $\pm$ 6.7	97.0-115.0	120.0-127.0			
Excretory pore	146.0 $\pm$ 14.1	116.0-172.0	125.0 $\pm$ 9.0	105.0-137.0	128.0 $\pm$ 5.1	123.0-134.0	131.0 $\pm$ 9.7	119.0-140.0	142.0-146.0			
Esophagus length	169.0 $\pm$ 14.4	141.0-192.0	159.0 $\pm$ 13.6	141.0-176.0	164.0 $\pm$ 6.4	155.0-169.0	165.0 $\pm$ 12.5	150.0-181.0	177.0-180.0			
Maximum body width	30.0 $\pm$ 3.0	24.0-35.0	26.0 $\pm$ 1.5	23.0-29.0	25.0 $\pm$ 1.7	23.0-27.0	29.0 $\pm$ 0.5	28.5-30.0	27.0-31.0			
Anal body width	23.0 $\pm$ 3.1	18.0-28.0	20.0 $\pm$ 1.1	18.0-22.0	17.0 $\pm$ 1.2	16.0-18.5	21.0 $\pm$ 1.1	19.0-22.0	19.0-25.0			
Tail length	58.0 $\pm$ 6.0	48.0-73.0	54.0 $\pm$ 3.4	47.0-60.0	63.0 $\pm$ 4.3	57.0-66.0	69.0 $\pm$ 6.6	62.0-80.0	65.0-76.0			
Tail annuli	33.0 $\pm$ 2.4	28.0-37.0	—	—	36.0 $\pm$ 2.1	34.0-39.0	40.5 $\pm$ 0.7	40.0-41.0	36.0-37.0			
Spicules	—	—	32.0 $\pm$ 1.3	30.0-34.0	—	—	—	—	—			
Gubernaculum	—	—	16.0 $\pm$ 1.2	14.0-18.0	—	—	—	—	—			



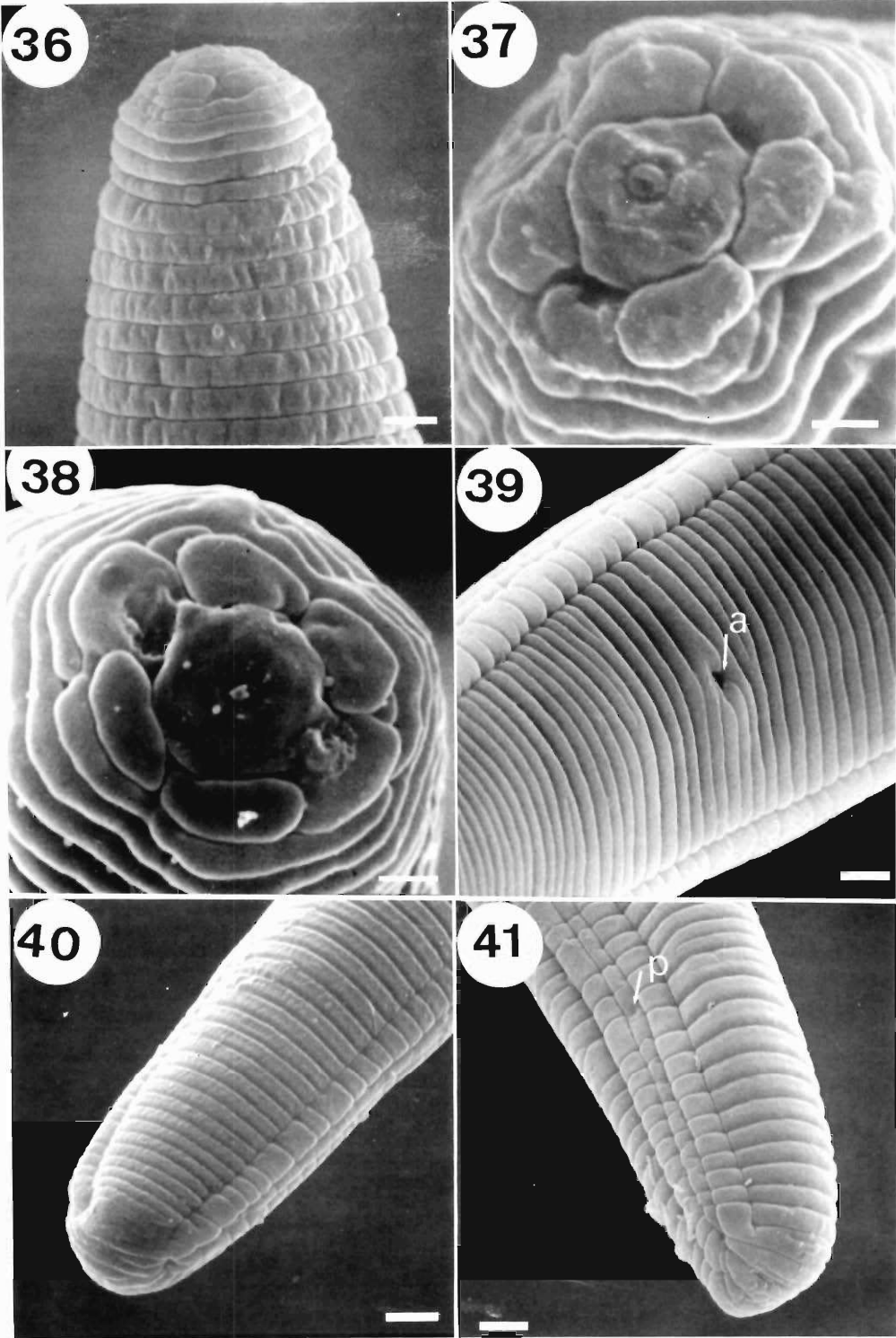


Figures 29–35. *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1986, from Spain. 29. Female anterior region. 30. Lip region. 31, 32. Female tails. 33. Spermatheca. 34. Mid-body region. 35. Male tail.

apolis (U.S.A.), coconut soil from Trinidad, and grass from Nottinghamshire, England.

**REMARKS:** This interesting species has a worldwide distribution being recorded from

U.S.A. (Allen, 1955; Thorne and Malek, 1968), Canada (Anderson, 1977), Pakistan (Maqbool and Shahina, 1987), Spain (S'Jacob et al., 1959; Zancada and Bello, 1981), Germany (Sturhan,



Figures 36–41. SEM micrographs of *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1986, from Spain. 36. Lip region. 37, 38. Face view. 39. Ventral view of anus. 40, 41. Tail termini. Scale bars: 36 = 2  $\mu\text{m}$ ; 37, 38 = 1  $\mu\text{m}$ ; 39 = 2.5  $\mu\text{m}$ ; 40, 41 = 5  $\mu\text{m}$ . a, anus; p, phasmid.

**Table 4.** Morphometric data for *Bitylenchus huesingi* (Paetzold, 1958) Siddiqi, 1986 (measurements in  $\mu\text{m}$ ).

	Females (N = 25)		Males (N = 3)	
	$\bar{x} \pm \text{SD}$	Range	$\bar{x} \pm \text{SD}$	Range
L	674.0 $\pm$ 62.4	581.0–847.0	700.0 $\pm$ 81.6	609.0–766.0
a	28.9 $\pm$ 2.3	25.2–35.3	32.0 $\pm$ 5.1	26.4–36.3
b	5.7 $\pm$ 0.4	5.0–6.5	5.5 $\pm$ 0.8	4.6–6.2
b <sub>1</sub>	10.2 $\pm$ 0.7	9.0–11.0	9.7 $\pm$ 1.7	8.0–11.3
V%	54.0 $\pm$ 1.5	51.0–57.0	—	—
T%	—	—	59.0 $\pm$ 4.0	55.0–63.0
G <sub>1</sub>	29.0 $\pm$ 5.1	20.0–35.0	—	—
G <sub>2</sub>	28.0 $\pm$ 3.5	22.0–34.0	—	—
c	13.7 $\pm$ 1.2	12.0–16.1	18.0 $\pm$ 1.4	16.9–19.6
c'	2.7 $\pm$ 0.3	2.4–3.3	2.2 $\pm$ 0.2	2.0–2.4
Stylet length	18.0 $\pm$ 1.0	16.0–20.0	17.7 $\pm$ 0.6	17.0–18.0
m	54.0 $\pm$ 2.7	50.0–59.0	56.0	—
o	13.0 $\pm$ 2.8	10.0–20.0	12.0 $\pm$ 0.7	11.0–12.0
S	1.3 $\pm$ 0.1	1.1–1.5	1.5	—
MB	51.0 $\pm$ 1.2	49.0–53.0	52.0 $\pm$ 1.7	50.0–53.0
Nerve ring	77.0 $\pm$ 7.5	67.0–90.0	85.0 $\pm$ 3.6	82.0–89.0
Excretory pore	94.0 $\pm$ 10.5	76.0–114.0	104.0 $\pm$ 6.7	96.0–108.0
Esophagus length	118.0 $\pm$ 10.4	99.0–135.0	127.0 $\pm$ 3.5	124.0–131.0
Maximum body width	23.0 $\pm$ 2.1	21.0–27.0	22.0 $\pm$ 1.7	20.0–23.0
Anal body width	18.0 $\pm$ 1.5	16.0–21.0	17.0 $\pm$ 1.5	15.0–18.0
Tail length	50.0 $\pm$ 5.1	39.0–59.0	39.0 $\pm$ 4.0	35.0–43.0
Tail annuli	47.0 $\pm$ 4.1	40.0–54.0	—	—
Spicules	—	—	26.7 $\pm$ 0.6	26.0–27.0
Gubernaculum	—	—	12.3 $\pm$ 0.6	12.0–13.0

1966), Netherlands (Dao, 1970), Poland (Brzeski, 1977), and France (Scotto La Massese and Du Merle, 1978). However, only 2 populations with females and males are known: from Canada (Anderson, 1977) and Spain (this paper). But there is an important difference between them because in Canadian females no sperm were found in the spermatheca of females whereas Spanish females were clearly inseminated with sperm. General morphology and measurements fit well with those given by Allen (1955) and others such as Thorne and Malek (1968), Anderson (1977), and Maqbool and Shahina (1987).

*Bitylenchus huesingi* (Paetzold, 1958)  
Siddiqi, 1986  
(Table 4)

**FEMALE:** Body ventrally curved upon relaxation. Lateral field 6–8 wide at mid-body, with 4 lines, outer bands regularly areolated. Lip region continuous with body, bearing 5–7 annuli and measuring 3–4 high  $\times$  6–7 wide. Stylet knobs rounded, slightly directed posteriad, 3–4 in diameter. Esophagus typical of the genus, with a slender isthmus 23–28 long. Excretory pore located opposite first third of basal bulb. Hemizonid 2–3 annuli long, located 3–5 annuli anterior to excretory pore.

Ovaries outstretched, equally developed. Tail straight, cylindrical, with annulated terminus. Terminal hyaline region 4–5 long. Postanal intestinal sac fills the tail cavity. Phasmids located 12–21 annuli posterior to anus level.

**MALES:** Uncommon. Similar to female in general morphology as well as measurements. Tail finely annulated, conoid. Spicules and gubernaculum ventrally curved. Bursa crenate, surrounds the tail completely, 64–70 long.

**HABITAT AND LOCALITY:** Pasture soil from Arroyo Frio, Sierra de Cazorla, Jaén, Spain.

**REMARKS:** Morphology as well as general measurements of this population are in close conformity with those given for the species by Paetzold (1958). It is well distributed in Europe where it has been recorded from Germany (Sturhan, 1966), France (Scotto La Massese and Du Merle, 1978), and Spain (Romero et al., 1970; Arias and Romero, 1971).

**Discussion**

**Remarks on the genus *Bitylenchus* Filip'ev, 1934**

*Bitylenchus* was proposed as a subgenus of the genus *Tylenchus* by Filip'ev (1934). It was synonymized with *Tylenchorhynchus* Cobb, 1913,

by Filip'ev (1936). Jairajpuri (1982) resurrected *Bitylenchus* as a subgenus of *Tylenchorhynchus* Cobb, 1913, and gave a key to its species. Siddiqi (1986) recognized it as a valid genus, gave a diagnosis to it, and differentiated it from *Tylenchorhynchus* "in having outer bands of lateral fields areolated, a large postanal intestinal sac containing intestinal granules and fasciculi, relatively more thickened cuticle at female tail tip, and a non-protrusible gubernaculum."

Fortuner and Luc (1987) rejected the genus *Bitylenchus*, arguing that it "was defined using very secondary characteristics that are not known for many taxa, and that, when known, do not clearly differentiate this genus from *Tylenchorhynchus*." They, however, assigned *Sauertylechus* Sher, 1974, a close relative of *Bitylenchus*, to the subfamily Belonolaiminae because, according to them, it "may be seen as a relict of ancestral forms from which evolved the present day belonolaimids," and because it "shares some derived characters with members of this subfamily (strong valve, elongate stylet)." We reject this apparently misleading "phylogeny" of *Sauertylechus* on grounds discussed below. The argument put forward by Siddiqi (1986) that this genus is similar to *Bitylenchus* except for the differences in the structure of the lip region is supported by our study of SEM face views of *Bitylenchus pratensis* sp. n. and *B. maximus* (Figs. 27, 37).

Sher (1974) stated that the type species *Sauertylechus labiodiscus* Sher, 1974, appeared similar to *Geocenamus* Thorne and Malek, 1968, in having a set-off lip region with the labial disc, a weakly developed cephalic framework, and a long thin stylet, but because it had 4 incisures (as against 6 in the latter), spicules with distal velum, a gubernaculum that protrudes from the cloacal aperture, and the absence of hypopygium, it could be placed in the genus *Tylenchorhynchus*. He placed *Sauertylechus* in the subfamily Tylenchorhynchinae, even though it had a lip region and stylet similar to the species of *Geocenamus* of the subfamily Merliinae and stated, "These two subfamilies are considered to be well defined (Siddiqi, 1971) after examining most of the species and many undescribed species in these taxa." Fortuner and Luc (1987) did not accept this statement and synonymized Merliniinae with Telotylenchinae because, according to them: 1) morphology of lateral field is not a primary differentiating character, 2) shapes of spicule and gubernaculum are accepted as generic characters only, and 3) the exact appearance of corpus, vul-

va, and spermatheca is not well defined in most species in the taxa concerned.

We concur with Sher (1974) and Siddiqi (1971) in regarding Merliniinae as a well defined group, separate from Telotylenchinae (which includes tylenchorhynchids). Those who have studied a large number of species in these groups can easily see that Merliniinae is distinct on the basis of the following characteristics: male and female genital structures (phylogenetically strong characters), such as spicules, gubernaculum, hypopygium, and shapes of vulva and spermatheca; SEM face views and the occurrence of 6 incisures in the lateral field; and presence of deirids (absence of deirids in *Scutylechus* is a derived character state in the group Merliniinae). In addition, and contrary to Fortuner and Luc's (1987) argument, *Merlinius* Siddiqi, 1970, in our opinion resembles *Amplimerlinius* Siddiqi, 1976, and both genera belong in Merliniinae, whereas *Tylenchorhynchus* resembles *Paratrophurus* Arias, 1970, and both belong in Telotylenchinae.

Fortuner and Luc (1987) assigned *Sauertylechus* and *Geocenamus* to Belonolaiminae, differentiating the latter from Telotylenchinae by the well marked round labial disc and "a tendency towards an elongation of the stylet to reach inside the roots." As discussed here, we regard *Sauertylechus* as a member of Telotylenchinae and *Geocenamus* as a member of Merliniinae. We believe that the development of a round labial disc is associated with the elongation of the stylet, the latter having occurred independently in various groups of Tylenchina. An SEM face view is required to see a round labial disc in some species, e.g., *Bitylenchus maximus*, *B. pratensis*, *B. velatus* (Sauer and Annells, 1981) comb. n. However, *Bitylenchus maximus*, with stylet 20.0–24.5, has a round labial disc, but *B. serranus* with stylet 19.0–22.5 as well as other *Bitylenchus* spp. with stylets under 19.0 such as *B. goffarti* (Sturhan, 1966) Siddiqi, 1986, and *B. tobari* (Sauer and Annells, 1981) Siddiqi, 1986, have a squarish labial disc, and annuli following it are broken by lateral indentations (Figs. 10, 63 and fig. 5B in Sher and Bell [1975]). In contrast, *Tylenchorhynchus* (see SEM face view of *T. cylindricus* Cobb, 1913, in fig. 5A in Sher and Bell [1975]) as well as *Paratrophurus* and *Histotylechus* Siddiqi, 1971, have complete labial annuli following a squarish labial disc.

We believe that Fortuner and Luc (1987) are unjustified in using the characteristics of the elongation of the stylet in the classification of these groups and would like to quote from Sid-

diqui (1986, p. 174) the following, to which we fully subscribe:

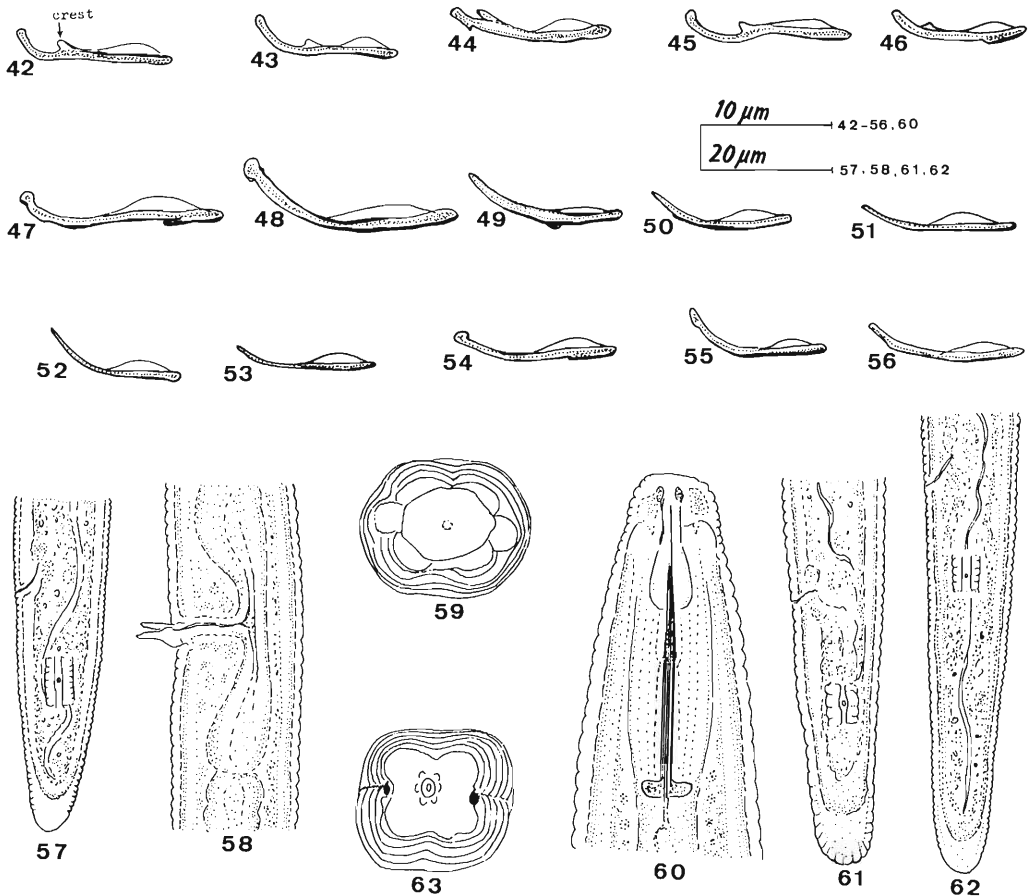
"The elongation of the stylet in Dolichodoriidae occurred independently in Dolichodoriinae, Macrotrophurinae, Tylenchorhynchinae (*Sauertylenchus*) and Merliniinae (*Hexadonrus*). Stylets with a solid-appearing tip (e.g. in *Tylenchorhynchus*, *Merlinius*, *Trophurus*), seldom reach a length over 30  $\mu\text{m}$ ; those with a tubular conus have a greater chance of exceeding this length. Stylet length can be used as a generic character with care, but it can not be the basis for grouping genera, e.g. *Dolichodorus*, *Belonolaimus* and *Macrotrophurus*, into one subfamily solely because they have excessively long stylets."

For rejecting *Bitylenchus*, Fortuner and Luc (1987) used some of the characters of *Bitylenchus* spp. that were, as it would appear from their discussion, based on published data and not actual examination of specimens. For example, they stated that a postanal intestinal sac was absent from *B. tobari* and *B. ventrosignatus* (Tobar Jiménez, 1969) Siddiqi, 1986. Upon examination of paratypes of these species, we found it to be present. In fact the sac extends into the entire tail cavity and contains intestinal granules and fasciculi (Figs. 57, 62). We also examined paratypes of *B. aerolatus* (Tobar Jiménez, 1970) Siddiqi, 1986, and found the species to belong to the genus *Tylenchorhynchus* to which it is here returned. In this species, there is no intestinal sac extending into the female tail and the gubernaculum has a winglike expansion in its proximal half (Fig. 44) different from the gubernaculum of all known species of *Bitylenchus*. We found this winglike expansion of the gubernaculum (crest) in several species of *Tylenchorhynchus* including the type species *T. cylindricus* Cobb, 1913 (Figs. 42–46). Because the crest is absent in *Bitylenchus*, it serves as a useful character in differentiating between the 2 genera. We have also found that the gubernaculum in several species of *Bitylenchus* is protrusible through the cloacal aperture when the spicules are protruded. A pair of pedunculated papillae are seen on either side of the cloacal aperture when spicules are protruded. A round labial disc and 6 sectors of the first cephalic annulus are seen in SEM face views of *B. pratensis* and *B. maximus* (Figs. 27, 28 and 36–41). A similar face view is seen in *T. velatus*. We examined paratypes of *T. velatus* and found that the species shows various characteristics of

*Bitylenchus*, including a postanal intestinal sac, typical gubernaculum, and modified vulva lips; hence, it is transferred to *Bitylenchus* as *Bitylenchus velatus* (Sauer and Annells, 1981) comb. n. In this and various other morphological characters (stylet and esophageal structures, genital structures, female tail having a large intestinal sac, etc.) these 3 species are similar to *Sauertylenchus labiodiscus* and should belong to 1 genus. However, at this stage we do not propose to synonymize *Sauertylenchus* with *Bitylenchus* but we regard it as a separate genus characterized by an elongated body (1.4–2.0 mm long), an offset labial disc, an elongated stylet measuring 33–40  $\mu\text{m}$  long in type species, and a characteristic gubernaculum that is distally recurved and has titillae. Because the characters of the labial disc and first head annulus being divided into 6 sectors are visible only by using SEM, and because these conditions are supposedly derived by the relative elongation of the stylet, we propose to retain *B. maximus*, *B. velatus*, and *B. pratensis* in the genus *Bitylenchus* and to emend the diagnosis of *Bitylenchus* in the light of the above discussion.

#### The genus *Bitylenchus* Filep'ev, 1934

Diagnosis (after Siddiqi, 1986; emended): Telotylenchinae: Small to medium size (0.4–1.5 mm long). Cuticle with fine but distinct annuli. Lateral field with 4 incisures, outer bands areolated. Deirids absent. Cephalic region with or without fine annuli, usually offset by a depression or constriction, and with lateral longitudinal indentations on annuli behind amphidial apertures. Labial disc with 6 labial sensilla usually roughly squarish in outline, but in some species round, and anteriormost lip annulus 6-sectored. Stylet attenuated, small, of medium strength, 10–24  $\mu\text{m}$  long; conus anteriorly attenuated and solid-appearing; knobs small, rounded, usually posteriorly sloping. Median esophageal bulb well developed, oval. Basal esophageal bulb offset from intestine, containing esophageal glands; cardia well developed. Vulva a small transverse slit, postmedian lips generally modified. Ovaries paired. Spermatheca round, axial. Postanal intestinal sac large, filling one-quarter or more of tail cavity, with intestinal granules and fasciculi. Female tail more than 2 anal body widths long, subcylindrical, cylindrical, or subclavate, with a rounded, usually striated terminus; terminal cuticle thickened but not excessively so. Male tail completely enveloped by a large, finely crenate



Figures 42-63. 42-46. Gubernacula in *Tylenchorhynchus*. 47-56. Gubernacula in *Bitylenchus*. 42. *Tylenchorhynchus cylindricus* Cobb, from U.S.A. 43. *T. brassicae* Siddiqi, paratype. 44. *T. aerolatus* Tobar Jiménez, paratype. 45. *T. mashoodi* Siddiqi and Basir, paratype. 46. *T. clarus* Allen, England. 47. *Bitylenchus maximus* (Allen), England. 48. *B. pratensis* sp. n., paratype. 49. *B. velatus* (Sauer and Annells, 1981) comb. n., paratype. 50. *B. goffarti* (Sturhan), Cyprus. 51. *B. indicus* (Siddiqi), paratype. 52. *B. teeni* (Hashim), paratype. 53. *B. colombianus* (Siddiqi) comb. n., paratype. 54. *B. botrys* (Siddiqi) comb. n., paratype. 55. *B. dubius* (Bütschli), England. 56. *B. parvus* (Allen), England. 57. *B. ventrosignatus* (Tobar Jiménez), paratype female, tail end. 58-61. *B. velatus* (Sauer and Annells). 58. Vulva region. 59. En face view taken from SEM by Sauer and Annells (1981). 60. Head end. 61. Tail end. 62. *B. tobari* (Sauer and Annells), paratype, tail end. 63. *B. tobari*, en face view taken from SEM by Sauer and Annells (1981).

bursa. Spicules distally flanged, 19-39 μm long; tip narrow, minutely rounded or indented. Hypopygium absent; a pair of pedunculated papillae are seen on either side of cloacal aperture when spicules are protruded. Gubernaculum large, 10-18 μm long, usually protrusible through cloacal aperture when spicules are protruded, distally boat-shaped in lateral view, and lacking crest (an expansion in distal half), telamon, or titillae.

*Type species:* *Bitylenchus dubius* (Bütschli, 1873) Siddiqi, 1986, syn. *Tylenchus dubius* Bütschli, 1873, *Tylenchorhynchus dubius* (Bütschli, 1873) Filip'ev, 1936.

*Other species* (for synonymies see Siddiqi, 1986): *B. botrys* (Siddiqi, 1985) comb. n., syn. *Tylenchorhynchus (Bitylenchus) botrys* Siddiqi, 1985; *B. colombianus* (Siddiqi, 1985) comb. n., syn. *Tylenchorhynchus (Bitylenchus) colombianus* Siddiqi, 1985; *B. pratensis* sp. n.; *B. seranus* sp. n.; *B. velatus* (Sauer and Annells, 1981) comb. n., syn. *Tylenchorhynchus velatus* Sauer and Annells, 1981.

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